BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES AND ITS EVALUATION AGAINST SOME FUNGAL PHYTOPATHOGENS

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BY

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INTRODUCTION

Nanotechnology is emerging as a broad field of study with its utilization in science and technology for constructing new materials at the nanoscale level. In general, most people accept that nanotechnology deals with structures usually in the range of 1-100 nanometers (nm) and involves developing materials or devices within that size. As nanoparticles exhibit unique chemical and physical properties, they have received much attention by the researchers working in many fields of science such as medicine, health, agriculture and environment etc. Nanotechnology has now started to revolutionizing the drug delivery sciences (Jain *et al.,* 2011). Silver nanoparticles have evoked interest in the past few years due to their antimicrobial properties (Choi *et al.,* 2008). They are even being projected as future generation antimicrobial agents (Rai *et al.,* 2009).

The word "nanotechnology" was introduced for the first time in a scientific literature by N. Taniguchi at "The International Conference on Industrial Production" in Tokyo in 1974 in order to describe the super thin processing of materials with nanometer accuracy and creation of nano-sized mechanisms. Ideas of nanotechnological approach, which were put forward by Feynman, were developed by E. Drexler in his book "Vehicles of creation: the arrival of the nanotechnology era" published in 1986.

Nanoparticles can be broadly grouped into two: namely organic and inorganic nanoparticles. Organic nanoparticles may include carbon nanoparticles (fullerenes) or produced from the dead or waste organic matter such as food materials *e.g.,* orange peel, potato peel etc. Some examples of the inorganic nanoparticles are magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles (like titanium dioxide and zinc oxide). Recently, biosynthetic methods employing either biological microorganisms such as bacteria and fungus or plant extract (Chandran *et al.,* 2006) have emerged as a simple and viable alternative to more complex chemical synthetic procedures for obtaining nanomaterials like copper, zinc, titanium, magnesium, gold, alginate and silver (Retchkiman-Schabes *et al.,* 2006). Silver nanoparticles have proved to be most effective as they possess antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms (Gong *et al.,* 2007). These nanoparticles possess unique properties, which find

myriad applications such as anticancer, antimicrobial, larvicidal, catalytic and wound healing activities.

Nanoparticles synthesized using biological systems are referred to as biogenic nanoparticles. The biological methods of nanoparticles synthesis using biological resources including bacteria, yeast, fungi (Ahmad *et al.,* 2002) and plants (Torresdey *et al.,* 2003) have already been reported as clean, innocuous and economically satisfactory ambient routes. The use of plant extracts for the synthesis of nanoparticles is potentially advantageous over microorganisms due to ease of scale up, less biohazard and elaborate process of maintaining cell culture. The biogenic synthesis of nanoparticles opens a new possibility of conveniently synthesizing pure metallic nanoparticles using natural products (Manisha *et al.,* 2013).

Fungi are responsible for more than 70% of all major crop diseases (Agrios, 2005) and significant crop loss is observed in several crop species like rice, wheat, barley, cotton, groundnut and grapevine (Dhekney *et al.,* 2007). Fungal infections are a major growing problem in plants. Crop cultivators continually battle with fungal diseases affecting their crops. Commercial agriculture relies heavily upon high inputs of chemical pesticides to protect crops against pathogens and pests. The resistance of pests and plant pathogens against pesticides and fungicides is rapidly becoming a serious problem. A range of broad-spectrum antibiotics, which made it possible to successfully treat infections of many fungi, had been found to be fatal in some cases. This resulted in prolonged survival of plants highly susceptible to fungal infection. In recent decades, bacteria and fungi have developed considerable resistance to many traditional and modern synthetic drugs. Plant pathogenic fungi must be controlled for the welfare of mankind. The antimicrobial properties of silver have been documented since 1000 B.C., when silver vessels were used to preserve water, but its applications in the field of agriculture have gained momentum very recently. Considerable researches has been performed on the *invitro* activity of silver nanoparticles (AgNPs) against several fungi, but very few researchers have paid attention to the application of AgNPs as antifungal compounds in controlling plant diseases in the fields and in turn promote the overall plant growth. Application of AgNPs in soil and as seed/seedling coatings may not only control the phytopathogen, but also

stimulate plant growth by several known and unknown mechanisms. In this context, nanoparticles (NPs) can also overcome the drug resistance mechanisms, related to decreased yield and productivity. Finally, NPs deliver the highest dose of antimicrobial agents specifically at the site of infection, thus overcoming drug resistance with less adverse effects on the plant.

In this regard, we will make an attempt to synthesize silver nanoparticles using seed extract of selected wild plants: *Citrullus colocynthis, Pedalium murex, Tribulus terrestris, Leucas aspera.* As the plant pathogenic fungi are gaining strong resistance against various insecticides and pesticides. To overcome this problem, nanoparticles will be biosynthesized from weeds in order to reduce the use of synthetic insecticides and pesticides. Thus for this purpose the antifungal activity of the prepared seed powder extract of the selected plants will be measured against selected plant pathogenic fungi: *Trichoderma harzianum, Fusarium oxysporum* f.sp. *lycopersici*, *Fusarium verticilloides, Fusarium sacchari.*

BIOSYNTHESIS OF NANOPARTICLES

The synthesis of metallic nanoparticles involves top-down and bottom-up approaches by chemical, physical, and biological means. Biogenic syntheses of silver nanoparticles are classified under bottom up approach (Sahoo, *et al.*, 2009). The use of plants for the synthesis of AgNPs has gained importance in the last decade because it is rapid, does not affect the environment, no pathogens are used and the whole process involves a single-step technique. Plant extracts contain a combination of biomolecules (*i.e.*, enzymes, polysaccharides, alkaloids, tannins, phenols, terpenoids and vitamins). Reduction of metal compounds into their respective nanoparticles is mediated by the microbial enzymes or the plant phytochemicals with antioxidant or reducing properties (Raveendran *et al.*, 2003).

The main concepts involved in the synthesis of AgNPs are:

- Appropriate solvent medium (either alcohol or water but preferably water).
- Eco-friendly reducing agent.
- Nontoxic stabilizer for the resulted nanoparticles.
- Ambient temperature and pressure, and neutral pH.

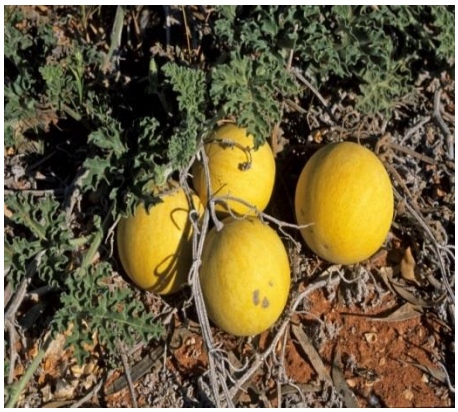
USE OF PLANTS TO SYNTHESIZE NANOPARTICLES

Plants have huge diversity thus can be used for wide range of applications in the field of agricultural, pharmaceuticals, industrial etc. Plants are being used as traditional medicines since ancient times as they are known to possess various medicinal and therapeutic properties. A wide range of plants has been currently investigated for their vital role in the synthesis of nanoparticles. Plants and their parts contain carbohydrates, fats, proteins, nucleic acids, pigments and several types of secondary metabolites which act as reducing agents to produce nanoparticles from metal salts without producing any toxic by-product. The plants are found to be advantageous over other resources because they are easily accessible, extremely easy and safe to handle. The use of plant and plant extract in nanoparticle synthesis is considered advantageous over microbial-based system because it reduces the elaborate process of maintaining cell cultures (Khatoon, *et al.,* 2017).

CHARACTERISTICS OF SELECTED PLANTS:

1. *Citrullus colocynthis* (L.) Schrad.

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Violales
Family:	Cucurbitaceae
Genus:	*Citrullus*
Species:	*colocynthis*



Citrullus colocynthis (L.) (Common name: bitter apple, bitter cucumber, desert gourd, and wild gourd) belongs to the family Cucurbitaceae. A fruit of *Citrullus colocynthis* is traditionally used for the treatment of diabetes, microbial diseases, ulcer, inflammation, jaundice and urinary diseases in Asian and African countries (Nmila *et al.,* 2000).

C. colocynthis has very high medicinal value; the plant contains three antitumor ingredients: cucurbitacin B, cucurbitacin E and D- glycoside of Beta- sitosterol (Rajkiran *et al.,* 2011). It has high

immune response mechanism by the production of secondary metabolites and thus it is useful to treat various bacterial and fungal diseases, which inhibits the growth of the plant.

2. *Pedalium murex* (L.)

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Lamiales
Family:	Pedaliaceae
Genus:	*Pedalium*
Species:	*murex*

Pedalium murex is a member of the sesame family, Pedaliaceae. It is found in different parts of the world such as Tropical Africa, Srilanka, India, Mexico and Pakistan. In India, it occurs mainly in the Western and Coromandal coasts as a weed of waste places and is generally called under the Hindi name "Gokhru or gokhar" and in Sanskrit as "gaja-daunstraka, gokshura" (Shukla *et al.*, 1983). *Bada Gokhru, Brihat Gokhru, Large caltrops, Peru-neranji, Kaitu nerinjil* are few common names of *P. murex*. It is a medicinal plant and used in Ayurveda, for treatment of inflammation, ulcers, fever and other disorders of genitourinary system and also possess various insecticidal and anti-microbial properties. *P. murex* is a good source of various bioactive constituents: flavonoids, tannins, alkaloids and phenolic compounds (Hill, 1952).

3. *Tribulus terrestris* (L.)

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Sapindales
Family:	Zygophyllaceae
Genus:	*Tribulus*
Species:	*terrestris*

Tribulus terrestris is an annual plant belongs to family Zygophyllaceae widely distributed around the world, which is adapted to grow in dry climate locations. Like many weedy species, this plant has many common names, including goat's-head, yellow Vine, chota gokharu, small caltrops, devil's-thorn, devil's-weed, puncture vine. *T. terrestris* is used in folk medicine as tonic, analgesic, diuretic, urinary anti-infective (Majeed & Mahmood, 1998) and is popularly claimed to improve sexual functions in man. *T. terrestris* showed antifungal and antibacterial activity because of the presence of active metabolites like saponins, phytosterols, alkaloids, etc. (Kostova and Dinchev, 2005 & Xu *et al.*, 2000).

4. ***Leucas aspera* (Wild.) Link**

Kingdom:	Plantae
Division:	Angiosperms
Class:	Dicotyledonae
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Leucas*
Species:	*aspera*

Leucas aspera commonly known as 'Thumbai'. It is distributed throughout India from Himalayas to Ceylon and in the plains of Mauritius and Philippines. It is typically found in dry, open, sandy soil. *L. aspera* is used commonly as an insecticide (Srinivasan, 2011). It is also used as an antipyretic; it is a herb that has the ability to help reduce fevers. The juice of the flowers can also be used for intestinal worm infections in children. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, and cytotoxic activity and as an antidote to snake venom.

Target pathogens

1. *Trichoderma harzianum*
2. *Fusarium oxysporum* f.sp. *lycopersici*

3. *Fusarium sacchari*
4. *Fusarium verticilloides*

1. ***Trichoderma harzianum* (Rifai):** *T. harzianum* is a fungus belonging to family Hypocreaceae. It is also used as a fungicide for foliar application, seed treatment and soil treatment for suppression of various disease causing fungal pathogens. They also exist in many other diverse habitats. *Trichoderma* spp. also attacks, parasitizes and otherwise gains nutrition from other fungi. It possesses high cellulolytic activity and main agent of decomposition. *T. harzianum* is responsible for green mould disease in mushroom (Jaylal *et al.*, 2007) and it is a major source of contamination and crop loss for mushroom farmers.
2. ***Fusarium oxysporum* f.sp. *lycopersici* (W.C. Synder & H.N. Hansen):** *F. oxysporum* f.sp. *lycopersici* is an ascomycetous fungus belonging to family Nectriaceae. It is a soil borne plant pathogen for the tomato plant which causes vascular wilt in tomato. The disease starts out as yellowing and drooping on one side of plant. Leaf wilting, plant stunting, browning of the vascular system, leaf death, and lack of fruit production are the symptoms of the disease (Wong, 2003). *F. oxysporum* f.sp. *lycopersici* pathogen enters in the plants through the roots and is spread throughout the plant by the vascular system.
3. ***Fusarium sacchari* (E.J. Buttler) W. Gams:** *F. sacchari* syn. *Giberella sacchari* (Summerell & J.F. Leslie) is a fungal plant pathogen belongs to family Nectriaceae. It is generally responsible for causing Pokkah boeng disease of sugarcane (Egan *et al.*, 1997) and is able to reduce the quality of the harvested crop especially amongst those varieties, which produces high sugar content. The pathogen is transmitted by air currents and air borne spores will colonize the leaves, flowers and stems of the plant (Martin *et al.*, 1961 and Burgess, 1981). Contaminated seed may spread the fungus. This fungus also has been recovered from orchids, sorghum etc.
4. ***Fusarium verticillioides* (Sacc.) Nirenberg:** *F. verticillioides* is the most commonly reported fungal species belonging to family Nectriaceae infecting maize (*Zea mays*). Earlier it was known as *Fusarium moniliforme*. It can be found in plant residues in almost every maize field

at harvest, yet the disease symptoms vary widely and range from asymptomatic infection to severe rotting of all plant parts. The infection of maize by this fungus can result in highly variable disease symptoms ranging from asymptomatic plants to severe rotting and wilting (Oren *et al.,* 2003). Environmental conditions, water availability, pathogen and the genetic background of the plant are the important factors in disease development.

REVIEW OF LITERATURE

Nanotechnology has the potential to revolutionize the different sectors of the agricultural and food industry with modern tools for the treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients, etc. Smart sensors and smart delivery systems will help the agricultural industry to combat viruses and other crop pathogens (Rickman *et al.,* 1999). In the near future, nano-based catalysts will be available which will increase the efficiency of pesticides and herbicides, allowing lower doses to be used.

Silver nanoparticles are the most studied and employed nanoparticles for biological system. These nanoparticles have been known for their strong bactericidal and inhibitory effects in addition to broad-spectrum antimicrobial activities. The studies on the applicability of nano-silver for controlling plant diseases has been limited till date (Lamsal *et al.,* 2011). Since the last decade, nanoparticle biosynthesis is the active area of research. Different biological entities such as plant, plant extracts, bacteria, fungi, and yeast have been explored for metal nanoparticle synthesis. Applications of nanotechnology in different fields like nano-food, nano-food packaging and nano-farming and also emphasized on nanoparticles and their effects on ecological balance. Plants or their extracts can be used in the synthesis of AgNPs as a green route. Such nanoparticles produced using plants have been used in various applications for human benefit. Elucidation of the mechanism of plant-mediated synthesis of nanoparticles is a very promising area of research.

S. No	Plant Extract Name	Size of Nano particle	Title of the Paper	Microorganisms Tested	Author and Year
1	Leaf extract of *Citrullus colocynthis*	-	Studies on antimicrobial efficiency of *Citrullus colocynthis* (L.) Schrad: a medicinal plant	*Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus*	Paul 2008
2	Leaf extract of	-	Antibacterial activity on	*Staphylococcus*	Gowri *et*

	Citrullus colocynthis		*Citrullus colocynthis* leaf extract	*aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Serratia marcescens*	*al.,* 2009
3	Leaf extract of *Citrullus colocynthis*	-	Antimicrobial activity of *Citrullus colocynthis* in Gulf of Mannar	*E.coli, Staphylococcus aureus, Klebseilla pneumoniae, Bacillus subtilis, Salmonella typhi, Aspergillus fumigatus, Mucor* sp., *Aspergillus flavus, Candida albicans*	Gurudeeban *et al.,* 2010
4	Leaf and fruit extract of *Citrullus colocynthis*	-	Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn.) Schrad against *Staphylococcus aureus*	*Staphylococcus aureus*	Najafi *et al.,* 2010
5	Calli extract of *Citrullus colocynthis*	-	Biomedical potential of silver nanoparticles synthesized from calli cells of *Citrullus colocynthis* (L.) Schrad	-	Satyavani *et al.,* 2011
6	Callus extract of *Citrullus colocynthis*	75nm	Green synthesis of silver nanoparticles by using stem derived callus extract of bitter apple (*Citrullus colocynthis)*	*E.coli, P. aeruginosa, Proteus vulgaris, L. monocytogens, Proteus mirabilis, Salmonella enteritidis,* and *Staphylococcus aureus*	Satyavani *et al.,* 2011
7	Leaf extract of *Citrullus colocynthis*	31nm	Plant mediated synthesis of Biomedical silver nanoparticles by using leaf extract of *Citrullus colocynthis*	*Escherichia coli, Bacillus subtilis, Proteus mirabilis, Salmonella enteritidis, Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa*	Satyavani *et al.,* 2011

8	Fruit extract of *Tribulus terrestris*	16–28 nm	Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: A novel biological approach	*Streptococcus pyogens, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus*	Gopinath *et al.*, 2012
9	*Ricinus communis*	29.18 nm	Biosynthesis of silver nanoparticles using *Ricinus communis* L. leaf extract and its antibacterial activity	*Bacillus fusiformis, Escherichia coli*	Singh *et al.*, 2012
10	Arial parts and fruits of *Citrullus colocynthis*	-	Antibacterial Activity of *Citrullus colocynthis* against different types of bacteria	*Escherichia. coli, Klebsiella pneumonia, Proteus mirabilis, Streptococcus pneumonia, Streptococcus agalactia, Streptococcus mutans* and *Staphylococcus aureus*	Bnyan *et al.*, 2013
11	Fruits, seeds, leaves and roots of *C. colocynthis*	7 – 19 nm	Green nanotechnology: Anticancer activity of Silver Nanoparticles using *Citrullus colocynthis* aqueous extracts	Colon adenocarcinoma (HCT-116), breast adenocarcinoma (MCF-7), liver carcinoma (Hep-G2) and intestinal adenocarcinoma (Caco-2)	Shawkey *et al.*, 2013
12	Fruits, seeds, leaves and roots extract of *Citrullus colocynthis*	-	Enhanced biocidal activities of *Citrullus colocynthis* aqueous extracts by green nanotechnology	*Staphylococcus aureus, Staphylococcus epidermidis Streptococcus pyogenes, Neisseria gonorrhoeae, Proteous vulgaris, Klebsiella pneumoniae, Shigella flexneri, Pseudomonas aeruginosa,Escherichia coli,*	Shawkey *et al.*, 2014

				Aspergillus fumigatus, Candida albicans, Geotricum candidum, Trichophyton mentagrophytes	
13	Plant extract of *Citrullus colocynthis*	-	Antimicrobial Activity of *Citrullus colocynthis* Extracts Against Soil Bacteria	Rhizosphere bacteria	Abdullah *et al.*, 2014
14	Leaf extract of *Tribulus terrestris*	15–40 nm	Synthesis, characterization and catalytic activity of silver nanoparticles using *Tribulus terrestris* leaf extract	-	Ashokkumar *et al.*, 2014
15	Flower extract of *Leucas aspera*	10-40 nm	Green Synthesis of Silver Nanoparticles Using *Leucas aspera*	-	Bharathi *et al.*, 2014
16	*Spirulina platensis*	12.73 nm	Green synthesis of silver nanoparticles using Single cell protein of *Spirulina platensis*	-	Verma *et al.*, 2014
17	*Vitis vinifera* fruit extract, Leaves extract of *Carica papaya*, *Nelumbo*, *Aloe vera* leaves extract, *Allium sativum* leaves extract, etc.	30-40nm, 25-40 nm, 25-80nm, 50-370 nm, 4-22 nm	A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise	*P. aeruginosa, P. mirabilis, E. coli, Shigella flexaneri, Klebsiella pneumonia, , S. aureus, A. flavus, A. niger, etc.*	Ahmed *et al.*, 2015
18	Fruits, root and leaves of *Citrullus colocynthis*	-	Phytochemical and antibacterial screening of *Citrullus colocynthis* of South-west Algeria	*Listeria monocytogenes, Bacillus stearothermophilus, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas*	Nora *et al.*, 2015

				aeruginosa, Escherichia coli	
19	Leaf extract of *Tribulus terrestris*	18-47 nm	*Tribulus terrestris* Leaf Mediated Biosynthesis of Stable Antibacterial Silver Nanoparticles	*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes, Proteus vulgaris* and *Bacillus subtilis*	Gopinath *et al.,* 2015
20	*Azadirachta indica , Eucalyptus, Thuja occidentalis, Cymbopogon , Ricinus communis, Beta vulgaris, etc.*	-	Plant and Plant Products for Silver Nanoparticles Synthesis: A Green Revolution in Nanobiotechnology	-	Satsangi *et al.,* 2015
21	Leaf extract of *Pedalium murex*	-	Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity	*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Micrococcus flavus, Pseudomonas aeruginosa, Klebsiella pheumoniae and Bacillus pumilus*	Anandalakshmi *et al.,* 2016
22	Leaf extract of *Leucas aspera*	-	Green synthesis of silver nanoparticles Green synthesis and analysis of silver nanoparticles Using *Leucas aspera*	*Bacillus cereus, Escherichia coli, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus and Yersinia*	Bilwashri *et al.,* 2016
23	Cauliflower extract, Papaya fruit extract, *Trianthema decandra* root extract, *Acalypha indica* leaf extract, *Withania somnifera* leaf extract, *Acorus calamus* extract,	42-83 nm, 15 nm, 10 nm, 20-30 nm. 5 - 30 nm. 31.83 nm	A review on use of plant extracts for gold and silver nanoparticle synthesis and their potential activities against food pathogens	*S. aureus, E.coli, A. niger, A. flavus, Candida albicans, Pseudomonas aeruginosa, etc.*	Antony *et al.,* 2016

	etc.				
24	Fruit, seed and Pulp extract of *Citrullus colocynthis*	ZnO-NPs 27–85 nm	Green Microwave-Assisted Combustion Synthesis of Zinc Oxide Nanoparticles with *Citrullus colocynthis* (L.) Schrad: Characterization and Biomedical Applications	*Bacillus subtilis, Staphylococcus aureus, Peseudomonas aeruginosa, Escherichia coli*	Azizi *et al.*, 2017
25	*Citrullus colocynthis*	-	Antimicrobial activity of methanol extract of *Citrullus colocynthis* against antibiotic-resistant *Staphylococcus aureus*	*Staphylococcus aureus*	Keikhaie *et al.*, 2017
26	Plant extract of *Leucas aspera*	25-80 nm	Biosynthesis of silver nano particles from *Leucas aspera* (willd.) Link and its anti-inflammatory potential against carrageen induced paw edema in rats	-	Kumaran *et al.*, 2017

Several reports are available on the biosynthesis of silver nanoparticles from plants, since few reports were available on the work of seeds of selected wild plants for checking its antifungal activity from the selected fungi. The seed powder extract which synthesizing most potent silver nanoparticles and showing maximum fungicidal effect among the four plants will be selected for further analysis.

OBJECTIVES

1. To collect the plant material from Bahadurpur site, Dayalbagh, Agra.
2. To screen the production of silver nanoparticles from seeds extract.
3. To characterize the silver nanoparticles (UV-Vis, XRD, EDX, FTIR, TEM, SEM).
4. To maintain the culture of targeted pathogenic fungi obtained from MTCC.
5. To study the antifungal activity of Silver nanoparticles in *invitro* and *invivo* conditions.

METHODOLOGY

1. **To collect the plant material from Bahadurpur site, Dayalbagh, Agra:** The seeds of the selected wild plants will be collected from Bahadurpur site, Dayalbagh, Agra. After collecting the seeds dried them in sunlight. Dried seeds of selected plants will be ground into fine powder. After that required amount of powder will be dissolved in distilled water and will be placed in water bath at 100^0C for boiling the mixture for about half an hour. After boiling, the sample will be allowed to filtered through Whatman's filter paper for obtaining the extract and then centrifugation will be done to remove further biomass and then will be stored in refrigerator (Banerjee *et al*., , 2011).

2. **To screen the production of silver nanoparticles from seeds extract:** 1mM, 2mM, 3mM concentrations of silver nitrate would be prepared in distilled water and will be used for synthesis of silver nanoparticles. Seeds extract will be added to each concentration of aqueous solutions of silver nitrate solution for reduction of Ag+ ions and will be observed for AgNPs at different time intervals over a period of 24 hours (Banerjee *et al.*, 2011).

3. **To characterize the silver nanoparticles (UV-Vis, XRD, EDX, FTIR, TEM, SEM):** With the help of UV-Vis spectrophotometer, we will monitor the reduction of pure silver ions by measuring the UV-Vis spectrum of the sample prepared. (Shankar *et al*., 2003). Further analysis of structure and composition of silver nanoparticles will be done by XRD. EDX will confirm the shape of nanoparticle and presence of elemental silver. FTIR analysis will confirm the bio reduction of Ag+ ions to silver nanoparticles, which is due to the reduction by capping material of plant extract. TEM analysis will determine the shape and size of silver nanoparticles. SEM will be performed for the detection of the size of the silver nanoparticles (Bar *et al*., 2009 & K. Mallikarjuna *et al*., 2014).

4. **To maintain the culture of targeted pathogenic fungi obtained from MTCC:** The plant pathogenic fungi (*Trichoderma harzianum, Fusarium oxysporum* f.sp. *lycopersici, Fusarium verticilloides,* and *Fusarium sacchari)* will be procured from MTCC. These obtained fungi will be maintained using SDA medium.

5. **To study the antifungal activity of Silver nanoparticles in *invitro* and *invivo* conditions:** The silver nanoparticles synthesis will be examined for their antifungal activity on the selected plant pathogenic fungi. Antifungal assay will be examined by measuring the minimum zone of inhibition by disc diffusion method (Vincent J. *et al*., 1944 and Perez C. *et al*., 1990) and taking pure seed extract and pure silver nitrate solution as control. The zone that will be obtained by the above method will prove the antifungal activity of silver nanoparticles synthesized from the seeds of selected plants and the one giving the maximum zone of inhibition will be selected for the further research.

   A pot experiment will be conducted to study the effect of biosynthesized silver nanoparticles on infected plants with selected fungal strains, followed by Kumar *et al.*, 2010 with some modifications. The developed nanofungicide will be evaluated for the treatment of diseased plant in *invivo* conditions. The methodology of checking the antifungal activity of biosynthesized silver nanoparticles is as:

   a) Healthy plants will be inoculated with selected fungal phytopathogens.
   b) After some time, disease development will be easily observed.
   c) Now, apply the developed nanofugicide of different concentrations on infected plants at different time intervals.
   d) Observations will be taken by studying the growth, yield and mortality evaluation. Healthy plants will be taken as control.
   e) Analysis of data and comparison of results with control.

SIGNIFICANCE

Nanotechnology is of great importance in today's world, development is the ongoing process and nanotechnology is adding on to this developmental process. Silver nanoparticles synthesized using nanotechnology is proving beneficial due to its vast characteristics in various fields, making it most widely used. Plant-mediated synthesis of AgNPs is more advantageous compared to the methods that use microorganisms especially because they can be easily improved, are less biohazardous and do not involve the elaborate stage of growing cell cultures. One of its most beneficial characteristic is the antifungal and antibacterial activity it shows against the various pathogenic fungi and bacteria.

The outcome of present research will be focus on the use of weeds for the synthesis of silver nanoparticles, to treat various diseases of plants caused by various pathogenic fungi. Pesticides help in the treatment of many diseases but over use of them are creating hazardous effects in the plants and they are developing resistance in them. Therefore, to control the misuse of pesticides, silver nanoparticles will be brought forward for treatment of various infectious diseases in plants caused by various fungi. Thus, in the present study an attempt will be made for the improvements in the efficacy of herbicides by nanotechnology could result in greater production of crops. The encapsulated nano-herbicides are relevant, keeping in view the need to design and produce a nano-herbicide that is protected under natural environment.

REFERENCES

Abdullah, S., Alshammary., Ibrahim, N.A., (2015). Antimicrobial activity of *Citrullus colocynthis* extracts against soil bacteria. *Glo. J. Bio. Agr. & Heal. Sci.* 3(4): 71-73.

Agrios, G.N., (2005). Plant Pathology. *Elsevier Academic Press*, San Diego, California, USA.

Ahmad, Mukherjee, P., Mandal, D., Sebapati, S., Khan, M.I., Kumar R., Sastry, M., (2002). Enzyme mediated extracellular synthesis of Cadmium silver nanoparticles by the fungus, *Fusarium oxysporum. Am. J. Chem. Soc.* 124: 12108-12109.

Ahmed, A., S., Ahmad, M., Swami, B.L., Ikram, S., (2015). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. J. Adv. Rese. 7: 17–28.

Albrect, M.A., Evans, C.W., Raston, C.L., (2006). Green chemistry and the health implications of nanoparticles. *Green Chem*. 8: 417-432.

Anandalakshmi, K., Venugobal, J., Ramasamy, V., (2016). Characterization of silver nanoparticles by green synthesis method using *Pedalium* murex leaf extract and their antibacterial activity. *Appl. Nanosci.* 6: 399–408.

Antony, E., Gunasekaran, S., Sathiavelu, M., Arunachalam, S., (2016). A review on use of plant extracts for gold and silver nanoparticle synthesis and their potential activities against food pathogens. *Asian. J. Pharm. Clin. Res.* 9(4): 18-23.

Ashokkumar, S., Ravi, S., Kathiravan, V., Velmurugan, S., (2014). Synthesis, characterization and catalytic activity of silver nanoparticles using *Tribulus terrestris* leaf extract. *Spectro. Acta Part A: Mol. & Biomol. Spectro.* 121: 88-93.

Azizi, Susan., Mohamad, R., Shahri, M.M., (2017). Green Microwave-Assisted Combustion Synthesis of Zinc Oxide Nanoparticles with Citrullus colocynthis (L.) Schrad: Characterization and Biomedical Applications. *Molecules.* 22: 1-13.

Banerjee, J., Narendhirakhannan, R.T., (2011). Biosynthesis of AgNPs from *Syzygium cumini* seed extract and evaluation of their *invitro* antioxidant activities. *Dig. J. Nanomat. & Biostr.* 6(3): 961.

Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., Pyne, S., Misra, A., (2009). Green synthesis of AgNPs using seed extract of *Jatropha curcus*. *J. Colloid. Surf.* 34(8): 212-216.

Bharathi, V., Vijaya anand, A., (2014). Green Synthesis of Silver Nanoparticles Using *Leucas Aspera. Int. J. for Pharma. Res. Sch.* 3(2): 524-526.

Bilwashri, H., Chebrolu, K.R., Ravindranath, B.S., (2016). Green synthesis of silver nanoparticles green synthesis and analysis of silver nanoparticles using *Leucas aspera. Int. J. Sci. & Eng. Res.* 3(1): 284-292.

Bnyan, I., Hasan, H., Ewadh, M., (2013). Antibacterial Activity of *Citrullus colocynthis* against different types of bacteria. *Adv. in Life Sci. & Tech.* 7: 48-51.

Burgess, L.W., (1981). General Ecology of the Fusaria. In P.E. Nelson, T.A. Toussoun and R.J. Cook (Eds.). *Fusarium: Disease, biology and taxonomy.* University Park and London: The Pennsylvania State University Press. 225-235.

Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A., Sastry, M., (2006). Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. *Biotechnol. Prog.* 22: 577-583.

Choi, O., Deng, K.K., Kim, N.J., Ross, L. J., Surampalli, R.Y., Hu, Z., (2008). The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res.*, 42: 3066–3074.

Dhekney, S., Li, A., Anaman, M., Dutt, M., Tattersall, J., Gray, D., (2007). Genetic transformation of embryogenic cultures and recovery of transgenic plants in *Vitis vinifera*,
Vitis rotundifolia and *Vitis hybrids*. *Acta Hort.*, 738: 743-748.

Egan, B.T., Magarey, R.C., (1997). Sugarcane. In R.J. Hillocks and J.M. Walker (Eds.). *Soilborne diseases of tropical crops.* Wallingford, Oxon, UK: CAB International.

Gong, P., Li, H., He, X., Wang, K., Hu, J., Tan, W., (2007). Preparation and antibacterial activity of iron and silver nanoparticles. *Nanotechnology.* 18: 604-611.

Gowri, S.S., Priyavardhini, S., Vasantha, K., Umadevi, M., (2009). Antibacterial activity on *Citrullus colocynthis* leaf extract. *Anc. Sci. of Life.* 29(1): 12-13.

Gopinath, V., Mubarak, A.D., Priyadarshini, S., Thajuddin, N., Velusamy, P., (2012). Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: A novel biological approach. *Coll. & Surf. B: Bioint.* 96: 69-74.

Gopinath, V., Priyadarshini, S., Venkatkumar, G., Saravanan, M., Mubarak A.D., (2015). *Tribulus terrestris* Leaf Mediated Biosynthesis of Stable Antibacterial Silver Nanoparticles. *Pharm. Nanotech.* 3(1): 000-000.

Gopinath V, MubarakAli D, Priyadarshini S, Meera Priyadharsshini N, Thajuddin N, Velusamy P (2012). Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: A novel biological approach. *Colloids and Surfaces B: Biointerfaces*. 96: 69-74.

Gurudeeban, S., Rajamanickam, E., Ramanathan, T., Satyavani, K., (2010). Antimicrobial activity of *Citrullus colocynthis* in Gulf of Mannar. *Int. J. of Curr. Res.* 2: 078-081.

Hill, A.F., (1952). Economic Botany. A textbook of useful plants and plant products. 2nd Edn. *McGraw-Hill book company*, Inc, New York.

Jain, S., Uchegbu, I.F., Betageri, G., Pastorin, G., (2011). Nanotechnology in Advanced Drug Delivery. *Journal of Drug Delivery*, 2011: 1-2.

Jaylal, R.G.U., Adikaram, N.K.B., (2007). Influence of *T. harzianum* metabolites on the development of green mould disease in the oyster mushroom. *Cey. J. Sci. (Bio. Sci.).* 36(1): 53-60.

Kahn, Jennifer., (2006). "Nanotechnology". *National Geographic*, 98–119.

Keikhaie, K.R., Ghorbani, S., Hosseinzadeh, Z., Hassanshahian, M., (2017). Antimicrobial activity of methanol extract of *Citrullus colocynthis* against antibiotic resistant *Staphylococcus aureus. Adv. Herb. Med*. 3(3): 1-6.

Kharissova, O.V., Dias, H.V.R., Kharisov, B.I., Perez, B.O., Perez, V.M.J., (2013). The greener synthesis of nanoparticles. *Trends in Biotechnology*. 31(4): 240-248.

Khatoon, N., Ahmed, J., Sardar, M., (2017). Biotechnological Applications of Green Synthesized Silver Nanoparticles. *J. Nanosci Curr. Res.* 2(1): 1-8.

Kumar, A., Prakash, P., Singh, N., (2010). *Maconellicoccus hirsutus* (Green) Infestation on *Jatropha curcas* L. Saplings and its possible management through herbal pesticides. http://www.scribd.com/doc/66036423/ICEP-4-Abstract.

Kumaran, N.S., Vijayaraj, R., Swarnakala., (2017). Biosynthesis of silver nano particles from *Leucas aspera* (willd.) Link and its anti- inflammatory potential against carrageen induced paw edema in rats. *Int. J. for Pharma. Sci. & Res.* 8(6): 2588-2593.

Lamsal, K., Kim, S.W., Jung, J.H., Kim,Y.S., Kim K.S., Lee, Y.S., (2011). Application of silver nanoparticles for the control of *Colletotrichum* species *in vitro* and pepper anthracnose disease in field. *J. Korean Soc. Mycolo. Mycobiol.,* 39: 194-199.

Leslie, J.F., Summerel, B.A., (2006). The Fusarium Laboratory Manual. John Wiley & Sons. 240-241.

Majeed, S.H., Mahmood, M.J., (1988). Herbs and medicinal plants in Iraq between traditional medicine and scientific research. 1st Ed. *Baghdad: Dar Al- Thaowra for Publishing*. 40.

Martin, J.P., Hong, H.L., and Wismer, C.A., (1961). Pokkah boeng. In J.P. Martin, E.V. Abbott, and C.G. Hughes (Eds.), *Sugarcane diseases of the world.* Amsterdam, The Netherlands: Elsevier Publishing Company. 1: 247-261.

Manisha, D.R., Kudle, R.K., Alwala, J., M, Anila., Sreedhar, B., Pratap, R.M.P., (2013). Synthesis of silver nanoparticles using extracts of *Securinega Leucopyrus* and evaluation of its antibacterial activity. *Int. J. Curr. Sci*. 7: 1-8.

K, Mallikarjuna., N, John Sushma., G. Narasimha., L. Manoj., (2014). Phytochemical fabrication and characterization of silver nanoparticles by using Pepper leaf broth, *Arab. J. Chem*. 7: 1099–1103.

Kostava, I., Dinchev, D., (2005). Saponins in *T. terrestris*- chemistery & bioactivity. *Phytochem. Rev.* 4(2): 111-137.

Nair, L.S., Laurencin, C.T., (2007). Silver nanoparticles: Synthesis and therapeutic applications, *J. Biomed. Nanotechnol*. 3: 301–316.

Najafi, S., Sanadgol, N., Nejad, B.S., Beiragi, M.A., Sanadgol, E., (2010). Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn.) Schrad against *Staphylococcus aureus. J. of Med. Plants Res*. 4(22): 2321-2325.

Nmila, R., Gross, R., Rchid, M., Manteghetti, M., Ribes, G., (2000). Insulinotropic effect of *Citrullus colocynthis* fruit extracts. *Plan. Med.* 66: 418-423.

Nora, N.B., Hamid, Kadi., Snouci, Moghtet., Boumediene, Meddah., Abdellah, M., (2015). Phytochemical and antibacterial screening of *Citrullus colocynthis* of South-West Algeria. *J. Chem. & Pharma. Res*. 7(5): 1344-1348.

Oren, L., Ezrati, S., Cohen, S., Sharon, A., (2003). Early Events in the *Fusarium verticillioides* - Maize Interaction Characterized by Using a Green Fluorescent Protein - Expressing Transgenic Isolate. *App.& Env. Microbio*. 69(3): 1695-1701.

Paul, J.P., (2008). Studies on Antimicrobial Efficiency of *Citrullus colocynthis* (L.) Schrad: A Medicinal Plant. *Ethnobot. Leaflets*.12: 944-47.

Perez, C., Pauli, M., Bazerque, P., (1990). An antibacterial assay by agar well diffusion method. *Acta. Bio. Et. Med. Exp*. 15: 113-115.

Rai, M., Yadav, A., Gade, A., (2009). Silver nanoparticles as a new generation of antimicrobials, *Biotechnol. Adv.* 27: 76–83.

Raveendran, P.F., Wallen, S.L., (2003). Green synthesis and stabilization of metal nanoparticles. *Amer. J. Advan. Chem. Sci.* 2(3): 208-211.

Rajkiran, C., Urjivia, G., Take, R.K., (2011). Medicinal importance of *Citrullus colocynthis*. *J. Chem*, 8(1): 85-90.

Retchkiman-Schabes, P.S., Canizal, G., Becerra-Herrera, R., Zorilla, C., Liu, H.B., Ascencio, J.A., (2006). Biosynthesis of characterization of Ti/Ni bimetallic nanoparticles. *Opt. Mater*. 29: 95-99.

Rickman, D., Luvall, J.C., Shaw, J., Mask, P., Kissel, D., & Sullivan, D., (1999). Precision agriculture: changing the face of farming. *Geotimes feature article.*

Sahoo, P.K., Kamal, S.S K., Kumar, T. J., Sreedhar, B., Singh, A. K., Srivastava, S. K., (2009). Synthesis of silver nanoparticles using facile wet chemical route. *Def. Sci. J*. 59(4): 447–455.

Satsangi, N., Preet, S., (2015). Plant and Plant Products for Silver Nanoparticles Synthesis: A Green Revolution in Nanobiotechnology. *Int. J. Emer. Res. Manag. & Tech.* 4(12): 25-29.

Satyavani, K., Ramanathan, K., Gurudeeban, S., (2011). Green synthesis of silver nanoparticles by using stem derived callus extract of bitter apple (*Citrullus colocynthis). Dig. J. of Nanomat. and Biostruc*. 6(3): 1019 -1024.

Satyavani, K., Gurudeeban, S., Ramanathan, T., Balasubramanian, T., (2011). Biomedical potential of silver nanoparticles synthesized from calli cells of *Citrullus colocynthis* (L.) Schrad. *J. Nanobiotech.* 26(9): 43.

Satyavani, K., Ramanathan, K., Gurudeeban, S., (2011). Plant mediated synthesis of biomedical Ag NPs by using leaf extract of *Citrullus colocynthis. Res. J. of Nanosci. & Nanotech.*1(2): 95-101.

Shawkey, A.M., Rabeh, M.A., K, A., Abdellatif, A.O., (2013). Green nanotechnology: Anticancer Activity of Silver Nanoparticles using *Citrullus colocynthis* aqueous extracts. *Adv. in Life Sci. and Tech.* 13: 60-70.

Shawkey, A.M., Abdulall, A.K., Rabeh, M.A., Abdellatif, A.O., (2014). Enhanced biocidal activities of *Citrullus colocynthis* aqueous extracts by green nanotechnology. *Int. J. of App. Res. in Nat. Pro.* 7(2): 1-10.

Shankar, S., Ahamad, A., Sastry, M., (2003). *Geranium* leaf assisted biosynthesis of Ag NPs. *Biotech. Progress.* 6(19): 1627-1631.

Shukla, Y.N., Thakur, R.S., (1983). Heptatriacontan - 4 - one tetratriacontanyl octacosanoate and other constituents from *Pedalium murex*. *Phytochem*. 22(4): 973-974.

Singh, A., Mittal, S., Shrivastava, R., Srivastava, J.N., (2012). Biosynthesis of silver nanoparticles using *Ricinus communis* L. leaf extract and its antibacterial activity. *Dig. J. of Nanomat. & Biostr.* 7(3): 1157 – 1163.

Siti, N., Nur, A.I., Azami, A.R., and Salleh, B., (2008). Distribution, morphological characterization and pathogenicity of *Fusarium sacchari* associated with Pokkah Boeng disease of sugarcane in Peninsular Malaysia. *Pertanika Jour. Trop. Agric. Sci.* 31(2): 279-286.

Srinivasan, R., (2011). *Leucas aspera*- Medicinal Plant: A Review. *Int. J. Pharma. & Biosci*. 2(1): 153-159.

Torresdey, G.J.L., Gomez, E., Peralta-Videa, J.R., Parsons, J.G., Jose, M., (2003). *Alfalfa* Sprouts: A natural source for the synthesis of silver nanoparticles. *Langmuir.* 19: 1357:1361.

Verma, S., Kumari, B., Shrivastava, J.N., (2014). Green synthesis of silver nanoparticles using single cell protein of *Spirulina platensis. Int. J. Pharm. Bio. Sci.* 5(2): (B) 458 – 464.

Vincent, J., Vincent., H., (1994). Filter paper disc modification of the Oxford cup *Penicillin* determination. *Proc .Soc. Exp. Biol. Med*. 4: 155-162.

Wong, Mui-Yun., (2003*). F. oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Synder and H.N. Hans. PP728 Soilborne Plant Pathogen Class Project, Spring.

Xu, Y.X., Chen, H.S., Liang, H.Q., G, Z.B., Lui, W.Y., Leung, W.N., Li, T.J., (2000). Three new saponins from *T. terrestris*. *Plan. Med.* 66(6): 545-550.